Research Article

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Reproductive Ecology of the European Pond Turtle, *Emys orbicularis* (Linnaeus, 1758), from Mediterranean Turkey

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Abstract: For two consecutive years (2012 and 2013), studies on reproductive ecology of *Emys orbicularis* were conducted at seven localities in the Mediterranean region of Turkey. Our findings show that individuals usually emerge from hibernation between the end of February and early March. Depending on latitude and elevation, mating occurred between March and May, and egg-laying lasted between May and July. Hatchlings were first observed between June and August. Average fecundity in females was 7 (5–10) eggs, and incubation period was determined to last between 80 and 110 days. Average size of eggs was 33.55 × 20.32 mm, and only a weak correlation between numbers of eggs and female body sizes (straight carapace length, SCL) was found. Average measurements of hatchlings were: SCL was 20.6 mm (18–23 mm), plastron length (PL) was 19.6 (17.6–22.4 mm) and weight was 3.6 g (2.9–4.3 g). These were compared to previously published results obtained for *E. orbicularis* from other parts of its range. Also, factors that affect reproductive success were discussed.

Key words: *Emys orbicularis*, breeding ecology, fecundity, Turkey

Introduction

The European Pond Turtle, *Emys orbicularis* (Linnaeus, 1758) (Testudines: Emydidae) is a reptile with a large distribution area ranging from North Africa over most of Europe and the Middle East to the Aral Sea (Fritz 2003). Like many chelonians throughout the world, the species is endangered and protected in many European countries (e.g. Fritz & Chiari 2013). At the international level, it is protected by the Bern Convention (1979) (Appendix II) and ANNEX II of the European Habitat and Species Directives (1992). Besides, The European Pond Turtle is regarded as Near Threatened in Europe and Vulnerable in the EU as a result of significant long-term population declines (Cox & Temple 2009). European populations of the species are threatened with degradation and loss of habitats, communal, industrial and agricultural water pollution, destruction of eggs though plowing, and occupying of their natural habitats by allochthonous freshwater turtles (Fritz & Chiari 2013). Thus, many populations seriously declined or they became extinct (Velo-Antón et al. 2008). Populations in Italy (Gariboldi & Zuffi 1994, Chelazzi et al. 2000), France (Cheylan & Poitevin 1998, Gay & Lebraud 1998), Poland (Mitrus & Zemanek 2004), Germany (Hanka & Joger 1998, Schneeweiss 2009), former Soviet Union (Shcherbak 1998, Kotenko 2000) and Turkey (Atatür 1995, Taşkavak & Reimann 1998, Ayaz et al. 2007, 2008) are endangered, especially because of habitat destruction. In recent years, morphology and phylogeography of the species have been studied in detail (e.g. Fritz 2001, 2003, Fritz et al. 2007, 2009), and studies of its biology and ecology picked up the pace. However, almost all of these studies were about the southern, central and northern European *E. orbicularis* populations.

The European Pond Turtle is distributed almost throughout Turkey (Baran & Atatür 1998, Fritz

2001) except in the east Black Sea region. The populations of the species in Central Anatolia are particularly dense (Ayaz, unpubl. data). Reproductive biology of the species has been a subject to analysis in various European populations and the current knowledge on reproduction of *E. orbicularis* was summarized by Fritz (2001, 2003). The status of populations of *E. orbicularis* is well known in many parts of Turkey (Ayaz, unpubl. data) and it has been a target species of conservation projects (Ayaz et al. 2013). In contrast, reproductive biology of *E. orbicularis* has not been well studied in the southern areas of the species' range (Ayaz & Çiçek 2011).

The present study provides data on clutch size, egg size, body size/clutch sizes relationship as well as nest site characteristic of *E. orbicularis* from Mediterranean region of Turkey.

Materials and Methods

The study was conducted in seven localities from the Mediterranean region of Turkey: (1) Kocagöl (Muğla), (2) Lake Gölhisar (Burdur), (3) Lake Eğirdir (Isparta), (4) Lake Suğla (Konya), (5) Anamur (Mersin), (6) Akgöl (Göksu River Delta, Mersin), and (7) Asi River Delta (Hatay) (Table 1, Fig. 1). The study covered three types of habitats: lake (1, 2, 3, 4), channel (5) and delta (6, 7) from the sea level (1, 5, 6, 7) to 1000 m a.s.l. (2, 3, 4).

The field studies were conducted in 2012 and 2013 (from beginning of February and to late November). In order to determine the reproductive phenology of the species, information about its reproductive behaviour, egg laying period and hatchlings were obtained during field studies. We attempted to determine the exact point of hibernation ending, the period of reproduction, and the periods of laying and hatching of the eggs. Clutch sizes were determined by direct observations in nests and with x-ray examination (15 mA, 70 kV, at 1 m distance) of females to determine presence of eggs and clutch sizes (Gibbons & Greene 1979). Furthermore, potential nesting sites were surveyed to determine nests position and the status of the nests were noted (vegetation, shape and slope). The captured turtles were marked individually by notching their marginal scutes (Ernst et al. 1974), and we measured: their weight (W) by a scale with 1 g accuracy; their straight carapace length (from the anterior edge of the carapace to the tip of the last posterior marginal scute, SCL); plastron length (from the outermost edge of the gular scute to the posterior end of the anal scute, PL); carapace height (CH) by a tortometer with 1 mm accuracy. Secondary sexual characters such as plastron concavity and tail length were used for distinguishing the sexes (Zuffi & Gariboldi 1995).

Normality of the SCL and PL distributions for sexes was tested with the Kolmogorov-Smirnov D test. Since they were normally distributed ($p \geq 0.05$), parametric t-test was used for their comparisons. The non-parametric Spearman's rank correlation was used to estimate the relationship between SCL, PL, and clutch size, as the data were

Fig. 1. Location of the surveyed localities: Kocagöl (1), Lake Gölhisar (2), Lake Eğirdir (3), Lake Suğla (4), Anamur (5), Akgöl (6), Asi River Delta (7)

Table 1. Surveyed localities

Locality	Province	Geographic position (coordinates)	Elevation (m a.s.l.)	Type of habitat	Number of processed turtles
Kocagöl (1)	Muğla	N 36°40'55.02" E 28°49'35.35"	8	Lake	–
Lake Gölhisar (2)	Burdur	N 37°6'57.17" E 29°35'56.99"	971	Lake	30
Lake Eğirdir (3)	Isparta	N 38°9'3.22" E 30°46'4.95"	1018	Lake	23
Lake Suğla (4)	Konya	N 37°19'31.31" E 32°0'18.18"	1092	Lake	17
Anamur (5)	Mersin	N 36°4'53.86" E 32°53'41.79"	Sea level	Channel	–
Akgöl (6)	Mersin	N 36°18'15.04" E33°57'3.63"	Sea level	Delta	5
Asi River Delta (7)	Hatay	N 36°3'45.40" E 35°58'48.03"	4	Delta	5

Table 2. Breeding phenology of *E. orbicularis* in the Mediterranean region of Turkey

Locality	Mating	Egg-laying	Hatching
Kocagöl (1)	March	May	June–July
Lake Gölhisar (2)	March–April	June	July–August
Lake Eğirdir (3)	April–May	June–July	July–August
Lake Suğla (4)	April–May	June–July	July–August
Anamur (5)	March	May–June	June–July
Akgöl (6)	March	May–June	June–July
Asi River Delta (7)	March	May–June	June–July

not normally distributed ($p \leq 0.05$). The alpha level was set at 0.05, and mean values are provided with their standard deviations. All statistical analyses were performed by the PAST statistical package (Hammer et al. 2001).

Results

Individuals generally emerge from hibernation at the end of February – beginning of March and, depending on the locality (latitude and altitude), mate between March and May; they lay eggs between May and July and hatchlings were first observed between June and August (Table 2). The earliest reproduction was observed in the Asi River Delta (Antakya) in early March and the latest at lakes Suğla (Konya) and Eğirdir (Isparta) in April–May.

According to our observations, females make nests in areas 5–200 meters away from water. They generally prefer areas near the shoreline. Nests are made at sites with slopes less than 45°, and are more often located near the roots of plants. Normally, places with vegetation consisting of *Nerium oleander*, *Vitex agnus agnus*, *Tamarix* sp., *Carex* sp., *Juncus* sp., and Poaceae members were preferred as oviposition sites. There were usually 1–3 nests near a plant root. The soil texture in the nesting area is sand, silt and clay areas; however, nests dug in harder soils were also observed. Females generally lay their eggs after sunset and at night, but sometimes they do this in the morning. During our study in Akgöl, only one individual was observed when laying eggs in the morning. The female first releases the cloacal waters in order to soften the ground, and then digs a hole with her hind limbs.

As females dig nest holes with their hind limbs, the opening of the nest is narrow, the base is wider and the nest is pear shaped (Fig. 2). The depth of a nest is approximately 8 cm and its width is app. 6 cm (n = 36). By taking the nesting time into consideration, the incubation period was estimated at 80–110 days. The shortest incubation period was observed in the Asi River Delta and the longest was at Lake Suğla.

In 2012, females from lakes Gölhisar (n = 10) and Eğirdir (n = 10) were radiographed in order to determine clutch sizes. Four individuals from Lake Gölhisar and two individuals from Lake Eğirdir had eggs (Fig. 3). The clutch size in these females counted between 5 and 9 eggs with an average of

Fig. 2. Nests from Lake Eğirdir (left) and Lake Gölhisar (right) (Photographed by Kerim Çiçek)

7 (SD = 1.67). In 2013, 60 females captured from Lake Gölhisar (n = 20), Lake Eğirdir (n = 13), Lake Suğla (n = 17), Akgöl (n = 5) and Asi River Delta (n = 5) were radiographed. Eggs were observed only in 23 of these females (Lake Gölhisar = 10, Lake Eğirdir = 7, Lake Suğla = 5, Asi River Delta = 1). Numbers of eggs observed in these females were 5–10 with an average of 7 (SD = 1.71).

During the whole study, eggs have been observed in 29 females (Lake Gölhisar: 12, Lake Eğirdir: 11, Lake Suğla: 5, Asi River Delta: 1). Mean SCL was 152.4 mm (range 132–171 mm, SD = 12.22), mean PL was 149.7 (128–165 mm, 16.14), and mean CH was 68.1 (62–72 mm, 4.09). We found that the number of eggs per female was between 5 and 10, calculated average was 7.3 (SD = 1.72). Average size of the eggs was calculated to be 33.55 mm (n = 46, SD = 2.61) and average width was calculated to be 20.32 mm (SD = 1.76). Weak positive correlation was determined between female body dimensions (SCL, PL, CH) and clutch size (Spearman's correlation coefficient, $r = 0.62$, $p < 0.05$ for SCL, $r = 0.52$, $p \leq 0.04$ for PL, $r = 0.63$, $p < 0.05$ for CH).

In hatchlings, mean SCL was 20.6 mm (n = 8, range = 18–23), mean PL was 19.6 mm (17.6–22.4) and mean weight was measured as 3.6 g (2.9–4.3) (Fig. 4).

During our studies we observed that nests were often plundered by predators like *Vulpes vulpes*, *Meles meles*, *Martes foina*, and *Lutra lutra.* Furthermore, some nests were destroyed near the Asi River Delta, Lake Gölhisar, Lake Eğirdir and Lake Suğla by agricultural activities like plowing. It is not possible to determine the destruction rate of the nests; however, it can be assumed that 50% of nets are destroyed at the studied localities in some years.

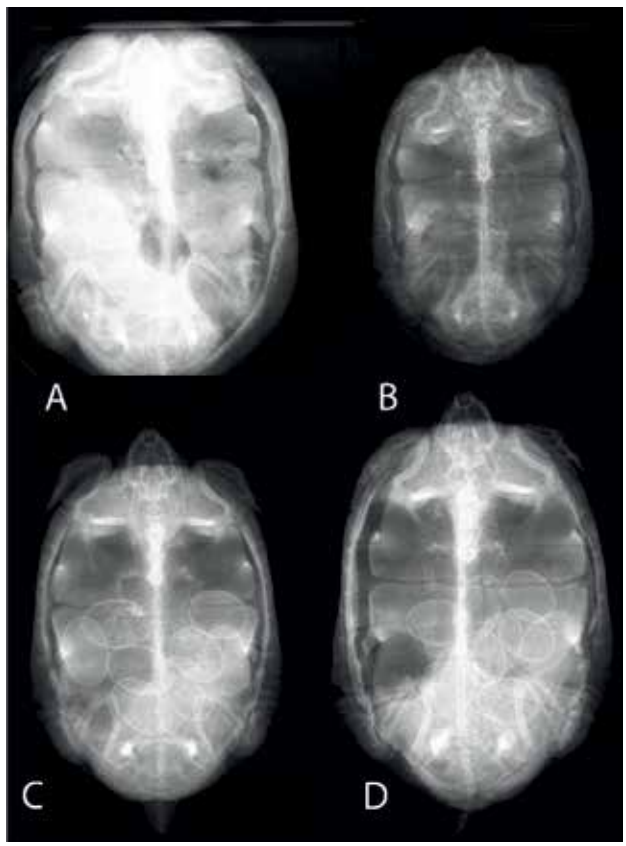

Fig. 3. Radiographed females from Lake Gölhisar [A. SCL = 17 cm, B. SCL = 13.8 cm, C. SCL = 15.1 cm, D. SCL = 17.1 cm]. There were no eggs in B

Discussion

The European Pond Turtle, *E. orbicularis,* is threatening throughout its range but the situation is strongly expressed on European populations. The species is faced with intensive habitat fragmentation and destruction especially due to urbanization pressure and agricultural activities in Anatolia. Besides,

Fig. 4. Captured hatchlings from Lake Eğirdir (Photographed by Kerim Çiçek)

ecological data on Asian populations are only anecdotal and inadequate for constructing effective conservation and management plans.

In Europe, *E. orbicularis* generally lays eggs between late May and mid-July. After approximately 74–117 days of incubation, hatchlings emerge, from August to late October, depending on latitude as well as on seasonal conditions (Lebboroni & Chelazzi 1991, Andreas & Paul 1998, Rössler 1999, Schneeweiss & Steinhauer 1998, Schneeweiss & Jablonsky 2000, Servan 1998, Fritz 2001, Novotný et al. 2004).

Geographic position and environmental conditions were shown to be the most important factors that determine body size and thus the reproductive ecology of *E. orbicularis* (Fritz 2001, 2003). Carapace length is generally used as the main predictive variable associated with clutch size and clutch mass variability (e.g. Gibbons 1982, Zuffi et al. 1999). Zuffi et al. (1999) reported that body size (especially CH) and clutch size are positively correlated. Body size is positively correlated with latitude; therefore, it is expected that females from northern populations are larger and produce larger clutches, while females from southern populations are smaller and thus have smaller clutches (Zuffi et al. 1999). Chelonians generally follow Bergmann's rule (body size is increasing with latitude, while decreasing with temperature) converse to squamates (Ashton & Feldman 2003). From the observed specimens, weak correlation was determined between the SCL, PL and CH with clutch size.

Clutch sizes were reported as following: 8–17 in Austria (Rössler 2000a), 4–11 in Hungary (Marián & Szabó 1961), 12 (6–16) in Slovakia (Novotný et al. 2004), 6–9 rarely 12 (Shcherbak & Shcherban 1980), and average 9–11 eggs (6–22) in Ukraine (Zinenko 2004). In Poland, average clutch sizes were 11 (5–17) (Najbar & Szuszkiewicz 2005), 15 (9–19) (Jablonski & Jablonska 1998) and the largest known clutch had 23 eggs (Mitrus & Zemanek 2000). In Mediterranean Turkey, clutches were generally smaller than in northern populations. Clutch sizes were 5–10 and the average was 7 eggs. The clutch size shows high variation in terms of latitude. Also, Schneeweiss (2004) emphasized that hatching success and sex ratio are affected by soil temperature and insolation duration at the nest site.

In some regions only one clutch is produced per season, e.g. Germany (Fritz & Günther 1996), Poland (Mitrus & Zemanek 2000) and Ukraine (Kotenko 2000). On the other hand, in some central and northern populations females usually have a second or even third clutch, e.g. in Hungary (Marián & Szabó 1961), Austria (Rössler 2000a, 2000b), Slovakia (Novotný et al. 2004), Belarus (Drobenkov 1999), Dagestan (Russian Federation) (Mazanaeva & Orlova 2004) and Poland (Najbar & Szuszkiewicz 2007). We were unable to determine whether females in our study localities lay eggs more than once per year.

Females generally lay eggs from near coastline (Shcherbak & Shcherban 1980, Mazanaeva & Orlova 2004) to 4 km away from water (Jablonski & Jablonska 1998). Mitrus (2006a) reported that the majority of turtle nests were located less than 150 meters from water bodies in central Poland. Females generally do not exhibit nesting site fidelity but few females lay eggs in particular nesting sites for long periods of time, thought fluctuations in environmental conditions (Mitrus 2006a, 2006b). The sex ratio may have male-biased due to the fact that vegetation grows and nesting areas become more shaded in central Poland (Mitrus 2006a).

Emys orbicularis shows successful embryonic development between 18 and 33°C (Pieau & Dorizzi 1981). Under laboratory conditions with constant incubation temperatures, the embryos develop male

characteristics at temperatures below 28°C and female characteristics above 29.5°C (Pieau & Dorizzi 1981, Schneeweiss 2004). Females predominate in Northeast Germany despite temperatures rarely exceeding 28.5°C in nests (Schneeweiss 2004). The incubation period was 74–89 days in Slovakia (Novotný et al. 2004), 75–90 days in Ukraine (Shcherbak & Shcherban 1980), 90–117 days in Austria (Rössler 2000a, 2000b), 85–113 days in Poland (Mitrus & Zemanek 2000), and 90–110 day in Dagestan (Mazanaeva & Orlova 2004). Incubation in Mediterranean Turkey completed in 80–110 days.

Most of the hatchlings emerge in August – September (Rössler 1999). In some populations hatchlings can overwinter in nest chambers and leave nests in the next spring (e.g. Mitrus & Zemanek 2000, Novotný et al. 2004). In our previous studies, we also observed overwintering behavior in some western Anatolian populations (Ayaz et al. 2007, Ayaz & Çiçek 2011). However, overwintering was not observed for Mediterranean populations.

Numerous terrestrial predators such as *L. lutra*, *V. vulpes*, *M. meles*, *M. foina*, *M. martes* and *P. lotor* occasionally predate upon European Pond Turtles, mainly their eggs (Fritz 2001, 2003, Lanszki et al. 2006). Mediterranean populations suffer from *L. lutra*, *V. vulpes* and *M. meles*. Besides ploughing and irrigation, other agricultural activities may destroy eggs. Especially, the *E. o. eiselti* population in Asi River Delta is strongly impacted by land cultivation.

In summary, the specimens of the Mediterranean Turkey population of the European Pond Turtle are smaller than the turtles in the northern regions of the geographical range of the species. The females from the investigated populations have smaller clutch size and the hatchlings do not overwinter. Agricultural activities and predators are the main threats for the nests of *E. orbicularis*. In conclusion, this study presents the first information about reproductive ecology of Anatolian populations of the European Pond Turtle *E. orbicularis*.

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